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High-Grade Dysplasia in Genital Warts From Two Patients Infected With the Human Immunodeficiency Virus

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Cancer-associated human papillomavirus (HPV) types are detected in genital warts removed from immunosuppressed individuals more commonly than from those occurring in otherwise healthy individuals. The prognosis of genital warts containing cancer-associated HPV types is not known. Because it is assumed that genital warts are benign lesions, they are usually treated by destructive therapies without prior knowledge of histopathology. The aim of the present study was to determine whether genital warts from individuals with or without human immunodeficiency virus (HIV) contain high-risk HPV types or areas of dysplasia. The study design was a nonrandomized analysis of genital warts removed by excision biopsy from 15 HIV-infected patients and 15 HIV-negative patients. The tissue was analyzed for HPV DNA by hybrid capture, and microscopic sections of each biopsy were examined for areas of dysplasia. Genital warts from HIV-infected patients contained cancer-associated (''high risk'') HPV types in 9 of 15 cases, including 1 that contained only a high-risk type. High-grade dysplastic abnormalities were present in 2 of the 15 lesions from this group, both of which contained high-risk HPV types. Four genital warts removed from HIV-negative patients contained high-risk HPV types, but none contained dysplastic abnormalities. It is concluded that genital warts from HIV-infected patients often contain high-risk HPV types. Such lesions may exhibit dysplastic changes. The frequency of dysplastic changes in genital warts from HIV-infected patients is not known. Biopsy of genital warts may be indicated prior to additional therapy in HIV-infected patients, and surgical removal should be considered as a preferred treatment option in these patients. ***J. Med. Virol. 54:69–73, 1998.***

KEY WORDS: human papillomavirus; dysplasia; genital warts

INTRODUCTION

Human papillomavirus (HPV) infection of the genital tract may be asymptomatic or may be manifested as a range of genital lesions, from genital warts to mildly dysplastic lesions to invasive carcinomas. Infections with certain genital HPVs, such as HPV types 6 and 11, cause external genital warts, a condition characterized by marked epithelial proliferation and a low risk of dysplastic changes. In contrast, other genital HPV types are highly associated with dysplastic cervical lesions. High-risk HPV types, such as HPV types 16, 18, and 31, are detected in up to 50% of genital warts removed from immunosuppressed individuals and occasionally from otherwise healthy individuals [Brown et al., 1994]. The risk of cancer in genital warts containing high-risk HPV types is not known. Because it is assumed that genital warts are benign lesions, they are usually treated by destructive therapies without knowledge of histopathology, regardless of the patient's immune status.

Individuals infected with the human immunodefi-

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ciency virus (HIV) have an increased risk of HPV infections and suffer from more severe consequences of such infections [Palefsky, 1994; Palefsky et al., 1993, 1994; Williams et al., 1994]. Most studies of HPV infection in patients infected with HIV have analyzed cytologic preparations from the cervical or anal mucosa rather than lesions on the external genitalia. In a previous study, external genital warts from 12 HIV-infected patients were examined for the presence of HPV DNA by using the hybrid capture assay [Brown et al., 1994]. Six of these 12 lesions contained both a high- and a low-risk HPV type. In contrast, both a high- and a low-risk HPV type were found in genital warts from 17% of control patients not infected with HIV. In the present study, biopsies of exophytic genital warts from 30 patients were analyzed to determine whether the presence of high-risk HPV DNA correlated with dysplastic changes. Fifteen of these patients, including the 12 described in the previous study, were infected with HIV.

MATERIALS AND METHODS

Study Population

Patients with typical external genital warts of the penis, vulva, or perianal area were identified in either a sexually transmitted diseases clinic or at a university-based clinic for care of individuals infected with HIV. HIV-negative control patients were chosen by identification of patients of the same sex and age as HIV-infected patients who had undergone treatment of genital warts. Overall, 11 male and 4 female patients were included in each group of HIV-infected and HIV-negative patients. The number of CD4 cells in the HIV-positive patients ranged from a high of 840/ml to a low of 10/μl. Most were receiving antiretroviral therapy, but the exact nature of the medications and dosages that several patients were receiving was not recorded at the time of biopsy. None was receiving protease inhibitors because these drugs were not available when the biopsies were obtained. Patients were not routinely cultured for the presence of other genital tract infections, such as herpes simplex virus.

Condylomata acuminata lesions (genital warts) were removed from the perianal region from 10 HIV-infected patients and from the penis (4) or vulva (1) in the remaining HIV-infected patients. For the HIV-negative control patients, 6 had perianal lesions removed, and the remaining were removed from the penis (8) or the vulva (1).

Lesions from 12 of the HIV-infected patients and 9 of the control patients were previously described in an analysis of HPV types in genital warts [Brown et al., 1994], but information regarding histopathology for all 30 patient lesions in this study has not been reported previously. All patients provided informed consent.

Biopsies of External Genital Warts

Biopsies of typical external genital warts were performed as previously described [Brown et al., 1992]. Briefly, exophytic lesions were cleaned with an iodine solution. Local anethesia was provided by intradermal injection of 1% lidocaine using a 25-gauge needle. Lesions were excised using sterile forceps and scissors. Bleeding was controlled by direct pressure with a gauze pad followed by electrocautery.

Samples were held in normal saline until processing occurred, which was generally within 2 hours. Biopsy samples were 2 mm^3 or larger and were split into two equal fragments. The first fragment, to be used to extract DNA, was frozen in liquid nitrogen. The second fragment was placed in zinc formalin to prepare paraffin-embedded sections.

Extraction of DNA

DNA was extracted from biopsy samples as previously described [Brown et al., 1994]. Briefly, biopsy samples were frozen with liquid nitrogen and then processed with a Braun mikro-dismembrator II (B. Braun Instruments, Melsungen, Germany). The resulting material was solubilized, treated with proteinase K, and extracted with phenol/chloroform/isoamyl alcohol. DNA was precipitated with ethanol and sodium acetate and quantified by spectrophotometry. The presence of high-molecular-weight DNA was established by agarose gel electrophoresis followed by staining with ethidium bromide. The yield from each tissue sample was approximately 10–50 μg of DNA.

Hybrid Capture Assay

The presence of HPV DNA by hybrid capture (Vira-Type Plus(R), Digene Diagnostics, Beltsville, MD) was detected as previously described [Brown et al., 1994] by using purified DNA from each genital wart. Briefly, RNA probes for 14 HPV types were allowed to hybridize under high stringency conditions to alkali-denatured specimen DNA. Positive specimens were detected by binding the hybridization reaction to tubes coated with a monoclonal antibody to RNA:DNA hybrids. Bound hybrids were detected by the addition of an alkaline phosphatase-conjugated antibody to RNA:DNA hybrids followed by addition of LumiPhos 530(R) and reading in an Optocomp I luminometer (MGM Instruments, Hamden, CT). The HPV probes used were divided into two pools whose composition is based on the association of each type with genital tract malignancy. Probe group A contained the low-risk HPV types 6, 11, 42, 43, and 44, and probe group B contained the high-risk HPV types 16, 18, 31, 33, 35, 45, 51, 52, and 56. Positive controls consisted of 1 pg of HPV 11 DNA (for probe group A) or 1 pg of HPV 16 DNA (for probe group B) diluted in 5 μg of HPV-negative DNA, each run in triplicate with each assay. One picogram of HPV DNA corresponds to 0.05 viral copies per cell in a sample containing 5 μg of cellular DNA. Patient samples were considered positive if the number of relative light units read from the luminometer was greater than the mean of the positive control values. The positive control had to be >1.5 times the negative control for the test to be considered valid.

TABLE I. Summary of Hybrid Capture Results From Genital Wart Biopsies[a]

	A+	B+	A/B+
HIV NEG (n = 15)	11	0	4
HIV POS (n = 15)	6	1	8

[a]A+, low-risk type detected; B+, high-risk type detected; A/B+, both types detected. HIV NEG and HIV POS represent patients in whom antibody testing for HIV was either negative or positive. A+ and B+ represent positivity of the hybrid capture assay for low-risk and high-risk HPV types, respectively (see text for HPV types included in the A and B probe groups).

Histopathology

One section from each sample was deparaffinized and stained with hematoxylin and eosin for histologic examination. All sections were examined by the same pathologist who was blinded to the HIV status of the patients and the HPV results.

RESULTS

HPV DNA was detected by hybrid capture in all 30 genital wart biopsies (Table I). All of the 15 genital wart biopsies from the control patients contained a low-risk HPV type, based on a reaction with the A probe group in the hybrid capture assay. Four of these biopsies also contained a high-risk HPV type (i.e., they also reacted with the B probe in the hybrid capture assay). No lesion from control patients contained only a high-risk HPV type. The mean (±SD) viral copy number for the low-risk HPV types in lesions from control patients was 3.9 ± 4.04 viral copies per cell (range = 0.58–12.20). The mean viral copy number of the high-risk types detected in lesions from control patients that were B probe positive was 0.21 ± 0.19 viral copies per cell (range 0.05 to 0.53).

A low-risk HPV type was detected in 14 of 15 samples from HIV-infected patients, and a high-risk HPV type was detected in 9 of 15 (Table I). One specimen contained only a high-risk HPV type. Southern blot analysis was performed as previously described [Brown et al., 1994] on the lesion containing only a high-risk HPV type, and a restriction pattern consistent with HPV 16 was detected (data not shown). The mean viral copy number was 5.45 ± 5.23 viral copies per cell (range = 0.08–14.90) for the low-risk HPV types in lesions from HIV-infected patients and 1.11 ± 2.68 viral copies per cell (range = 0.06–8.2) for high-risk HPV types. In each case, samples negative for that probe group were excluded from the means.

Detection of high-risk HPV types in genital warts from HIV-infected patients was compared with that of control patients by using a chi-squared distribution. Although there was a trend toward an increase in detection of high-risk types in HIV-infected patients, this was not a statistically significant value ($P = 0.066$). The mean HPV copy numbers in the genital warts biopsies did not differ significantly between HIV-infected and control patients for low- or high-risk types (one-tailed t-test, $P = 0.19$ for low-risk HPV types, $P = 0.17$ for high-risk HPV types).

Histopathological examination revealed features typical of genital warts including papillomatosis, hyperkeratosis, and epithelial acanthosis in all 30 biopsy samples. Two samples from HIV-infected patients had high-grade dysplastic changes seen on biopsies. One of the two lesions showing high-grade dysplasia contained only a high-risk HPV (1.1 viral copies/cell) and was a perianal lesion (Fig. 1). In this lesion, there were areas of immature keratinocytes growing in a disorganized fashion and a degree of residual epithelial maturation with overlying koilocytotic atypia. However, in other areas, the epithelium was completely replaced by more primitive basaloid cells with numerous mitotic figures scattered throughout the epithelium. This lesion was described as severe dysplasia. The second high-grade dysplastic lesion (not shown) contained a low- and a high-risk HPV type (1.25 and 0.6 viral copies/cell, respectively). This lesion was removed from the penis and was described as containing features typical of condylomata acuminata with focal moderate Bowenoid dysplasia. No biopsy from an HIV-negative patient contained areas of high-grade dysplasia.

DISCUSSION

Many studies have demonstrated the benign nature of genital warts, which are most often caused by HPV types 6 and 11. Certain HPV types, such as 16, are associated with dysplastic changes of epithelial tissues, especially the uterine cervix [zur Hausen and Rosl, 1994].

Biopsies of clinically diagnosed genital warts from immunosuppressed patients who have received kidney or liver transplants often contain more than one HPV type, including those types associated with genital tract malignancies [Brown et al., 1994]. Dysplastic changes may be associated with the presence of high-risk HPV in genital warts from this group of patients [Brown et al., 1994]. In the present study, excision biopsies were performed on typical genital warts that were not suspected of containing dysplast ic cells. Both cases of high-grade dysplasia in genital warts from HIV-infected patients contained high-risk HPV types. One biopsy contained only a high-risk HPV type; in the second case, both a high- and a low-risk HPV type were detected.

The frequency of high-grade dysplasia in genital warts of the penis, vulva, or perianal areas in patients infected with HIV is not known, and the present study is too small to answer this question. Previous studies have found cases of dysplasia in genital warts from HIV-infected patients and otherwise healthy individuals. HIV-infected patients with genital warts are at increased risk for anal squamous cell carcinoma [Daling et al., 1987; Palefsky, 1994]. Bradshaw et al. [1992] described an HIV-infected patient whose genital wart contained HPV 16 and dysplastic abnormalities. Biopsy of the lesion demonstrated superficial cells with perinuclear halos and nuclear atypia. Areas of full-thickness cytological atypia were present, with atypical

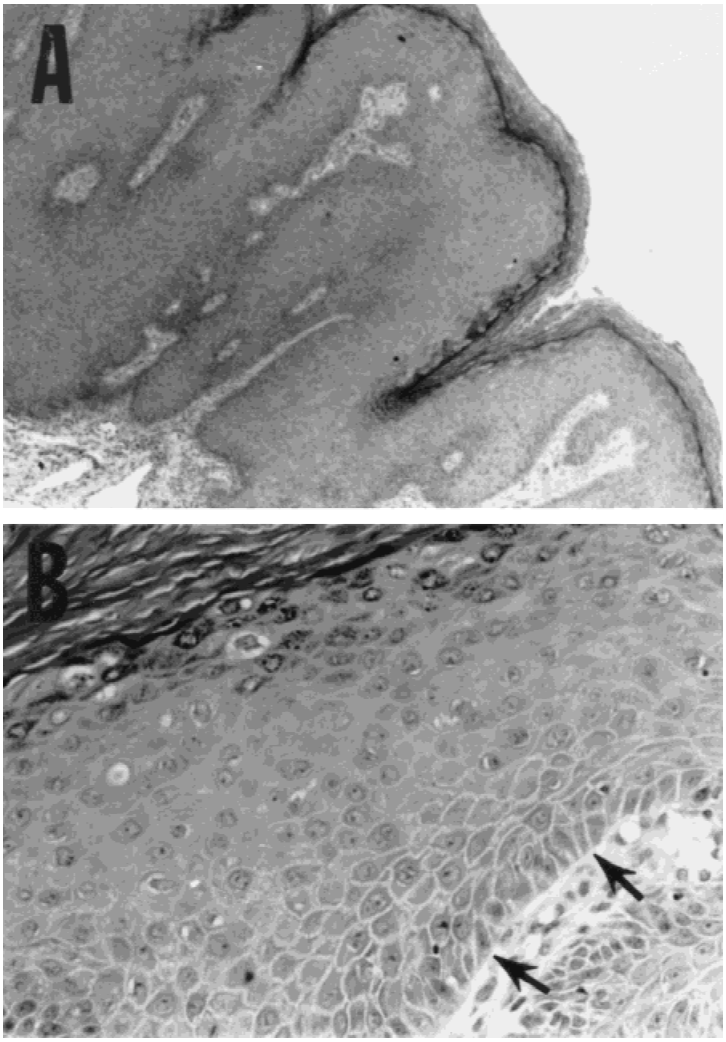

Fig. 1. Histopathology of genital wart biopsy from an HIV-infected patient. This lesion contained only high risk HPV DNA. The perianal biopsy contains areas of high grade dysplasia. There is a full thickness epithelial abnormality characterized by variable epithelial hyperplasia. The epithelium is composed of immature keratinocytes that are growing in a disorganized fashion. Focally, there is some degree of residual epithelial maturation with overlying koilocytotic atypia. However, in other areas the epithelium is completely replaced by more primitive basaloid cells. Numerous mitotic figures are noted scattered throughout the epithelium. Atypical mitoses are also seen. The underlying dermis demonstrates a mild focally moderate chronic inflammatory response. There is no evidence of invasive cancer. In some adjacent areas of the biopsy, the cytoarchitectural features are a lower grade and more closely resemble a typical genital wart. This area of condylomatous architecture merges with the severe dysplasia. Original magnification: Panel A: 100 ×; Panel B: 400 ×. Arrows in Panel B indicate the basal layer of cells.

mitotic figures, and dysplastic cells consistent with squamous cell carcinoma in situ.

Bernard et al. [1992] analyzed penile and perianal genital warts from HIV-infected male patients and uninfected control patients. HPV DNA was detected by in situ hybridization. HPV types associated with high risk of genital tract malignancy, including HPV types 16, 18, 31, 35, and 51, were detected in 83.4% of genital tract biopsies, including 36% of genital warts lacking areas of dysplasia. Potentially oncogenic HPV types were detected in the majority of lesions with features of intraepithelial neoplasia.

Demeter et al. [1993] analyzed 44 male patients with penile intraepithelial neoplasia (PIN) and 88 male patients with condylomata acuminata lesions. The HIV status of the population was not known. Patients with PIN or genital warts free of dysplastic abnormalities could not be distinguished on the basis of clinical presentation. The authors concluded that PIN may be more common than has been previously recognized because biopsy of lesions suspected to be genital warts is not a part of routine clinical practice. In that study, pigmented genital lesions were more often PIN than nonpigmented lesions, although 43% of PIN grade III lesions were not pigmented.

Other studies have suggestd that HPV 16 and other high-risk HPV types are rarely present in genital warts [Gupta et al., 1987; Syrjanen et al., 1987; Duggan et al., 1989]. Most of these studies relied on DNA in situ hybridization methods for detection of HPV sequences. DNA in situ hybridization studies only examine a portion of the lesion and may not detect small foci of in-

fection with a different HPV type. Sensitive methods, such as hybrid capture that use total DNA extracted from the biopsy, may be more likely to detect such foci of HPV 16-containing cells. Even more sensitive tests, such as consensus primer polymerase chain reaction followed by type-specific hybridization, may permit identification of high-risk types in a higher percentage of patients than were identified in our study [Shah et al., 1997].

A larger study of HIV-infected patients is needed to determine the true prevalence of high-risk HPVs in genital warts and dysplastic abnormalities. Patients are routinely treated without first determining the histopathology of the lesion. Knowledge of tissue pathology could influence treatment in HIV-infected individuals whose lesions contain high-risk HPV types because determining the boundaries of dysplastic lesions may be necessary for prevention of recurrence and disease progression. In addition, close follow-up of patients with high-grade dysplasia in genital wart biopsies seems prudent.

An excision biopsy may be preferable to other treatment modalities in immunosuppressed patients. Excision biopsy provides histologic data regarding the tumor margins and is well tolerated, even in HIV-infected patients with many exophytic lesions. Commonly used methods of treatment such as cryotherapy do not provide information about tumor margins and, in our experience, are no better tolerated than excision biopsy. In addition, excision biopsy is safe and simple to perform. Recurrence of genital warts is common, but there are limited data regarding relative recurrence rates with different forms of therapy. Excision biopsy, however, is associated with a low rate of recurrence [Bonnez et al., 1996].

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